

Improved Neurodevelopment Following Antiretroviral Therapy in HIV-Infected Children

Laurén A. Gómez, BS,¹ Claudia S. Crowell, MD, MPH,² Irene Njuguna, MBChB, MSc, MPH,³ Lisa M. Cranmer, MD, MPH,⁴ Dalton Wamalwa, MBChB, MMed, MPH,³ Daisy Chebet,³ Vincent Otieno, MBChB, MPH,³ Elizabeth Maleche-Obimbo, MBChB, MMed, MPH,³ Melissa Gladstone, MBChB, MRCP, MRCPCH, MD,⁵ Grace John-Stewart, MD, PhD,¹ and Sarah Benki-Nugent, MS, PhD¹

¹Dept. of Global Health, Univ. of Washington, Seattle, WA, USA; ²Dept. of Pediatrics, Div. of Pediatric Infectious Diseases, Univ. of Washington and Seattle Children's Hospital, Seattle, WA, USA; ³Dept. of Paediatrics & Child Health, Univ. of Nairobi, Nairobi, Kenya; ⁴Dept. of Pediatrics, Emory Univ. and Children's Healthcare of Atlanta, Atlanta, GA, USA; ⁵Dept. Of Women's and Children's Health, University of Liverpool, Liverpool, UK

Reprints or Correspondence Dr. Sarah Benki-Nugent: Dept. of Global Health, Univ. of Washington, Box 359909, 325 9th Ave., Seattle, WA 98104; Phone: 206-543-4278; Email: benki@u.washington.edu; Fax: 206-543-4818

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ABSTRACT

Background Late HIV diagnosis following severe co-morbidity remains common in resource limited settings. Neurodevelopmental recovery during antiretroviral therapy (ART) for late-diagnosed children is understudied. We determined 6-month neurodevelopmental trajectories in HIV-infected children initiating ART during hospitalization. **Methods** HIV-infected children initiated ART following HIV diagnosis during hospitalization in Kenya. The Malawi Developmental Assessment Tool (MDAT) was administered after clinical stabilization within 1-month and at 6-months post-ART initiation. Baseline versus 6-month Z-scores for each developmental domain were compared; cofactors for change in z-scores were evaluated using linear regression. **Results** Among 74 children, median age was 1.7 years (interquartile range, 0.8, 2.4) and median z-scores for gross motor, fine motor, social, and language domains were -1.34, -1.04, -0.53, and -0.95, respectively. At baseline, children with higher plasma viremia had lower social z-scores ($P=0.008$). Better nourished (weight-for-age z-score [WAZ] ≥ -2) children had higher z-scores in all developmental domains (all P -values ≤ 0.05). After 6 months on ART ($n=58$), gross and fine motor z-scores improved significantly (mean change 0.39; $P=0.007$ and 0.43; $P=0.001$, respectively), but social and language did not. Children with better immune and growth response to ART had higher gains in gross motor (0.05 per unit-gain CD4%, $P=0.04$; 0.34 per unit-gain WAZ; $P=0.006$ and 0.44 per unit-gain height-for-age z-score (HAZ); $P=0.005$), social (0.37 per unit-gain WAZ; $P=0.002$) and language (0.25 per unit-gain HAZ; $P=0.01$). **Conclusions** Children had significant neurodevelopmental gains during 6-months of ART, and children with better growth and immune recovery had greater improvement. Prompt commencement of ART may improve neurodevelopment in addition to immunity and growth.

Key words child development, nutrition, Africa, Malawi Developmental Assessment Tool

ACCEPTED

INTRODUCTION

Globally, 1.8 million children are HIV-infected, most of whom reside in sub-Saharan Africa (1). With improved survival (2, 3) it is important to optimize long-term growth, development, health and quality of life. Early HIV diagnosis and treatment are essential for the best outcomes (4, 5). However, late HIV diagnosis remains common in sub-Saharan Africa (6-9). In 2015, UNAIDS estimated that only 49% of HIV-infected children were receiving antiretroviral therapy (ART) (1).

HIV-infected children have high risk of neurodevelopmental compromise. In historical birth cohorts of HIV-infected infants with either no or limited ART, 26-36% of children had motor or cognitive delay by age 24 months (10-12). In long-term treated pediatric cohorts, learning difficulties and low cognitive scores have been common (13-15). HIV-related cognitive impairment likely results from a local inflammatory cascade involving small molecules, cytokines and chemokines, that can damage neurons and is driven by low-level viral replication and recruitment of activated immune cells to the brain (16). Abnormalities in white matter microstructure and subcortical gray matter are linked to cognitive deficits in children and adolescents (17-20). Children with greater severity of HIV disease prior to ART have worse cognition compared with asymptomatic children (13, 21, 22).

For infants who start ART within the first few months of life, cognitive compromise may be minimal. Early-treated infants in South Africa had similar one-year neurodevelopmental ability as HIV-uninfected infants (5). The extent to which ART benefits neurodevelopment in older children is less clear. Few studies have prospectively followed children from ART initiation. School-aged Thai and Cambodian children did not have cognitive improvements during their first 3 years of ART (23), and similarly, South African children (median age, 5

years), did not have improved cognitive performance 6 months after initiating ART (24).

However, in the Democratic Republic of Congo, children with median age 44 months had significant 1-year gains in both motor and cognitive scores after initiating ART (25).

We characterized neurodevelopmental trajectories and cofactors for change among HIV-infected children age <6 years and initiating ART after hospitalization in Kenya. We hypothesized that children with better response to ART would have higher neurodevelopmental gains during ART.

METHODS

Study Population

This study was nested within the Pediatric Urgent Start of Highly Active Antiretroviral Treatment (PUSH Study) randomized clinical trial (RCT) of urgent (ART at <48 hours) versus post-stabilization (ART at 7-14 days) ART (NCT02063880) (n=191) (26). Ethical approval was obtained from the University of Washington (UW) Institutional Review Board, the University of Nairobi/Kenyatta National Hospital (KNH) National Hospital Ethics and Research Committee, the Pharmacy and Poisons Board, and the Ministry of Health, Kenya. From 2013-2015, hospitalized HIV-infected children were identified at KNH and Mbagathi District Hospital in Nairobi and Jaramogi Oginga Odinga Teaching & Referral Hospital and Kisumu East District Hospital in Kisumu, Kenya. Routine HIV testing was conducted as described previously (9). Inclusion criteria for the parent RCT were: HIV-positive, <12 years of age, and ART naïve [other than antiretrovirals used for prevention of mother to child transmission (PMTCT)]. Children were excluded from the parent RCT if they had evidence of, or suspected, central nervous system infection. At enrollment and scheduled follow-up visits, children had a clinical exam and developmental and growth assessments. All children in both treatment arms, who were

clinically stable, and who were within the age inclusion criteria for the Malawi Developmental Assessment Tool (MDAT) (age <5.5 years to accommodate the 6 month follow-up) were eligible to receive neurodevelopmental assessments. Weight, height and head circumference were determined using a calibrated weighing scale, a measuring rod and a non-elastic measuring tape. Blood specimens were collected at enrollment and week 24 for hemoglobin, plasma HIV RNA, and CD4 count and percentage.

Per 2011 Kenyan guidelines, children >3 years or >10 kilograms (kg) received abacavir (ABC) plus lamivudine (3TC) plus nevirapine (NVP) or efavirenz (EFV). Children <3 years or <10kg received ABC plus 3TC plus NVP or ritonavir-boosted lopinavir (LPV/r) if previously exposed to NVP as part of PMTCT (27). Starting September 2014, all children <3 years were started on LPV/r-based regimens regardless of NVP exposure, in accordance with national guideline changes (28).

Developmental Assessments

Assessments were performed using the MDAT, which has been validated and demonstrated to have good sensitivity and specificity in rural and urban African settings (29). A standardized script for each item was developed, which was translated to Kiswahili and back-translated to ensure accuracy prior to study start. Clinicians administered items in either English or Kiswahili depending on caregiver and child preference. Assessments were performed following clinical stabilization, at either 2 or 4 weeks post-ART (baseline) and at 24 weeks (6 months) post-ART initiation. The MDAT assesses gross motor, fine motor, social, and language domains, with 34 pass/fail items in each. Cognitive skills are assessed within the language and fine motor domains. Most gross and fine motor and language items must be directly observed, and some may be caregiver reported. All social items may be caregiver reported. Items were administered until a

child had 6 consecutive passes and 6 consecutive fails. Domain specific raw scores were calculated using the number of passes, assuming passes for items preceding the 6 consecutive passes. Raw scores were converted to z-scores using norm data for healthy children in rural and urban Malawi (n=1445) and collected by MG (29).

Laboratory Testing

Laboratory assays were performed at the University of Nairobi and Centers for Disease Control and Prevention/Kenya Medical Research Institute (CDC/KEMRI) in Kisumu. HIV RNA was quantified using the Abbot Real-time HIV-1 Assay (Abbott Molecular Inc., Des Plaines, IL). CD4+ T cell count and percentage were determined using a FACSCount (Nairobi) and a FACSCalibur (Kisumu).

Statistical Analysis

Differences in baseline characteristics for children with and without baseline developmental data were compared using Wilcoxon rank sum and chi-square tests for dichotomous and continuous measures, respectively. Z-scores for weight-for-age (WAZ), height-for-age (HAZ), weight-for-height (WHZ) and head-circumference-for-age (HCZ) were calculated using the WHO child growth standard reference population (30). Underweight, stunting, wasting and microcephaly were defined as z-score <-2 for WAZ, HAZ, WHZ and HCZ, respectively. Dichotomized variables were used to evaluate severe immunosuppression (CD4% <15%), pre-ART viremia (HIV RNA >10⁶ copies/mL [c/mL]), and post-ART lack of virologic suppression (HIV RNA >10³ c/mL) (31). Cofactors for baseline developmental z-scores were evaluated using univariate and multivariate linear regression models. Primary cofactors of interest were HIV disease parameters (CD4% and plasma HIV RNA level) and nutritional status (WAZ and HAZ). Sociodemographic characteristics and potential confounders (listed below) were also evaluated.

Baseline and 6-month developmental z-scores were compared using paired t-tests. Cofactors for the magnitude of change in developmental z-scores from baseline to 6-months were evaluated using univariate and multivariate linear regression models adjusting for baseline developmental z-scores. Primary cofactors of interest were baseline HIV, growth parameters, and 6-month change in CD4%, viral suppression (plasma HIV RNA $<10^3$ c/mL), and change in WAZ and HAZ. Sociodemographics (child age and sex, caregiver age, marital status, and education, and household rent, people per room and one-room house), pre-term birth (self-report), and receipt of PMTCT were also evaluated. Final models for the magnitude of change outcomes were adjusted for baseline developmental z-score, child sex and caregiver years of education. These variables were selected based on associations in the univariate models at significance $P<0.1$. Variables that were collinear with cofactors of interest were not included in multivariate analyses. Sensitivity analyses excluding children with history of pre-term birth were also evaluated and results were generally similar except where noted. All analyses were performed using Stata SE version 13.1 (Stata Corp., College Station, Texas, USA). Study data were collected and managed using REDCap electronic data capture tools hosted at the UW Institute of Translational Health Sciences (32).

RESULTS

Cohort Characteristics at Baseline

Among 74 children <5.5 years of age with baseline MDAT data (Figure 1), the median enrollment age was 1.7 years (25th, 75th percentiles, 0.8, 2.4) (Table 1). Nineteen percent of children had received PMTCT. Median CD4% was 18% (25th, 75th percentiles, 11, 23). Most (61.6%) children had a WHO Stage diagnosis of 3 or 4. Most (91.9%) had at least mild anemia (hemoglobin <11 g/dL) and 23.0% had severe anemia (hemoglobin <7.0 g/dL) (33). Most were

underweight (WAZ <-2; 54.1%) or stunted (HAZ <-2; 58.1%). Nearly all children (93.2%) were cared for by their biological mother, and all had breastfed. Nine percent of caregivers reported having pre-term birth. Children who did not have an MDAT assessment (most often due to death or being too ill; Figure 1), were more likely to be underweight (77.0% vs 54.1%, $P=0.003$), younger (medians, 1.3 vs 1.7, $P=0.05$), immunosuppressed (CD4% medians, 13.4 vs 17.8, $P=0.04$), WHO stage 3 or 4 (78.7% vs 61.6%, $P=0.02$) and from the Nairobi catchment site (60.8% vs 39.5% $P=0.009$).

Baseline Developmental Functioning

At baseline, median developmental z-scores for gross motor, fine motor, social and language domains were -1.34, -1.04, -0.53, and -0.95, respectively. Children with poorer HIV disease severity indicators and nutritional status had lower baseline developmental scores (Figure 2 and Table, Supplemental Digital Content 1, <http://links.lww.com/INF/C994>). Children with higher plasma HIV RNA log₁₀ had lower social scores (regression coefficient (RC) or mean decrease per unit HIV RNA increase, -0.34, $P=0.008$), with similar trends for gross and fine motor ($P=0.07$ and $P=0.06$). Children who were underweight or who had stunting had lower scores in all domains (all P values ≤ 0.05), and results were similar in models adjusted for baseline CD4% and plasma virus level. Children with microcephaly had significantly lower gross motor scores (mean difference, -1.29; $P=0.003$). Children whose caregivers reported pre-term birth had lower developing functioning in gross motor, fine motor and social domains (mean differences, -1.06, $P=0.03$; -1.08, $P=0.03$; and -1.12, $P=0.004$, respectively).

Developmental Functioning at 6 Months Post- ART Initiation

Developmental assessments were available for 58 children at 6-months post-ART initiation (Figure 1). After 6 months of ART, gross motor (mean change, 0.39; standard deviation (SD),

1.01; $P=0.007$) and fine motor (mean change, 0.43; SD, 0.92; $P=0.001$) z-scores improved significantly, while social and language did not (mean changes, 0.11; SD, 1.06; $P=0.4$ and -0.20 ; SD, 0.65; $P=0.03$, respectively) (Table 2).

Children with better baseline immune and nutritional status (weight, height and head circumference) had greater improvements in developmental functioning after 6 months on ART (Figure 3 and Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C995>). In multivariate analysis adjusting for baseline developmental z-scores, child sex and caregiver years of education, children with baseline CD4% <15% had lower gains in social scores (mean difference in gain, -0.62 ; $P=0.02$), (Figure 3 and Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C995>). Children beginning ART with higher baseline stature had greater gains in gross and fine motor, social and language. For each one-unit increase in baseline HAZ, there was a 0.21 mean gain in gross motor z-score ($P=0.02$), with similar results for other domains. Children with larger baseline head circumference had greater gains in fine motor (mean gain 0.26 per unit increase in HCZ; $P=0.001$) and social (mean gain 0.21; $P=0.02$). Older children had higher gains in social z-scores (mean gain, 0.22 per year increased age; $P=0.03$).

During ART, children with higher gains in weight, height, and CD4% had greater improvement in gross motor (mean gain, 0.34 per gain in WAZ; $P=0.006$, 0.44 per gain in HAZ; $P=0.002$, and 0.05 per gain in CD4%; $P=0.04$), social (mean gain, 0.37 per gain in WAZ; $P=0.002$) and language (mean gain, 0.25 per gain in HAZ; $P=0.01$), (Figure 3 and Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C995>).

DISCUSSION

Among newly diagnosed HIV-infected children age <5.5 years, we found significant gains in gross and fine motor domains, but not in social or language during 6 months on ART. Children

who were better nourished, had lower viremia and better immune status at baseline started with higher baseline developmental z-scores. During ART, children with taller baseline stature, and better immune and growth recovery while on ART had greater developmental improvements.

During the first 6 months on ART, children in our study had substantial gains (approximately +0.4 SDs) in gross and fine motor, whereas social and language did not improve. These gains in motor function may reflect reduced virus or inflammation within the brain or improved overall health or both. A previous study among similarly aged (median, 44 months) African children with late HIV diagnosis, found substantial motor and cognitive improvements after initiating ART (25). However, South African children initiating ART at mean age 60 months and with severe immunosuppression had no neurodevelopmental improvement during 6 months on ART (24). In another study, South African infants (mean age 5 months) had limited improvement, with no gain in language or motor and trend for improved cognition during 6 months of ART (34). Our finding of significant improvement following ART in an older cohort suggests residual neuroplasticity in response to viral suppression and immune recovery and underscores the role of prompt ART to prevent cognitive decline.

The first 2 years are critical for brain development (35). Several mechanisms, including irreversible HIV-related neuronal damage, HIV brain reservoirs refractory to ART, and undernutrition may limit neurodevelopmental benefits of ART. In our study, children with advanced HIV had poorer developmental scores at presentation. Other studies have also shown that children with HIV disease progression had greater memory and learning deficits (36), psychiatric (22) and cognitive impairment (13, 21, 22, 37) cortical atrophy, and white matter microstructural changes indicative of neuronal and myelin injury (17, 18, 38).

We found that children with poor nutritional status had significantly lower baseline MDAT scores. Undernutrition is a major risk factor for poor neurodevelopment (39). Children in this study had substantial undernutrition compared with the general population in Kenya, in whom underweight, stunting and wasting were 11.0%, 26.0% and 4.0%, respectively, in 2014 (40). Undernutrition in this cohort may have reflected combined food insecurity, and the metabolic cost of HIV and acute co-morbidities.

Given that late perinatal HIV diagnosis continues to be common in sub-Saharan Africa (6-9), it is important to identify cofactors of better neurodevelopmental recovery in this context. In US cohorts, children with viral suppression prior to age 5 had higher school-age IQ scores (41). We found that children with better pre-ART immune and nutritional status, particularly as indicated by height, and those with better immune and growth responses to ART had higher developmental gains. These findings suggest that nutritional support in newly diagnosed HIV-infected children might benefit their developmental trajectories.

Even under optimized circumstances, successful ART may not prevent neurodevelopmental impairment in HIV-infected children. In adults, evidence of neuronal damage can persist even in the context of effective viral suppression (42). Among very early treated infants, a subset still manifested brain abnormalities by age 32 months (43). During 6 months on ART, we did not find improvements in either social or language functioning, and z-scores remained approximately 0.5 SDs below the norm for both domains. Importantly, lack of improvement in these domains may precede long-term deficits that compromise behavior, functioning, school achievement and employment. School aged HIV-infected children often have deficits in processing speed, working memory, visual-spatial processing, attention, language and executive functioning (37, 44-48).

In our cohort, mean baseline developmental z-scores were ~0.5-1 SD below a Malawian norm and though improved significantly in some domains, remained below average after 6 months of ART. Our findings are similar to a South African cohort (mean age 5 months) whose developmental scores remained significantly lower than HIV-exposed uninfected infants after 6 months of ART (34). In randomized trials, psychosocial stimulation improved neurodevelopment in HIV-infected children on ART in Uganda (mean age 3.8 years) and in South Africa (mean age 18 months) (49, 50). Further development and scale-up of interventions to augment ART are needed to optimize child outcomes in perinatally HIV-infected children.

To our knowledge, our study is the first to use the MDAT, a publicly accessible tool that was culturally adapted for use in Africa, for serial developmental assessment of HIV infected children (29). The MDAT can be administered in 20-60 minutes and may be a useful tool for screening and monitoring HIV-infected infants in HIV-care settings. Unlike the Denver Developmental Screening Test, the MDAT provides a score that can be used to compare to local norms, and monitor for improvements or declines over time. Additional studies are needed to determine the utility of the MDAT for scaled-up assessment of child developmental outcomes in HIV care settings.

Strengths of this study are the sample size, prospective design, use of a detailed developmental assessment, and inclusion of HIV-infected children newly initiating ART. Children were HIV diagnosed in hospital, which unfortunately remains common (6-8). This study used a detailed developmental assessment, which allowed quantitative group comparisons, and may have greater potential for scale-up than methods requiring more intensive training and resources.

Limitations include the relatively short follow-up, and high attrition prior to first developmental assessment. In addition, we lacked detailed information regarding likely key determinants of early childhood development including history of obstetric or birth complications, duration of breastfeeding, and the home caregiving environment. History of pre-term birth was limited due to self-report and lack of ultrasound in this setting. The MDAT is less widely validated than other assessments, such as the Bayley Scales of Infant and Toddler Development, which has been used in several other studies of infant neurodevelopment in HIV-infected children (10-12, 14, 15, 34). We did not evaluate developmental trajectories in an age-matched HIV-unexposed Kenyan cohort. Lack of change in language and social z-scores may have been due to low sensitivity in these domains or subtle differences in language, culture or environmental and psychosocial contexts, given that this analysis relied on Malawian norm data. The MDAT is currently being used in other African countries (personal communication, M. Gladstone); however, validation studies for use of MDAT in these settings have not yet been published.

In conclusion, HIV-infected children with late diagnosis had improved gross and fine motor functioning during the first 6 months on ART, no improvement in social or language functioning and overall, scores remained below African norms. Immune and nutritional status were correlated with developmental improvement, suggesting that optimizing nutrition and ART may improve neurodevelopmental benefits. Residual deficits underscore the need for further interventions to optimize neurodevelopmental outcomes in late-diagnosed HIV-infected children.

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FIGURE LEGENDS

Figure 1. Flow chart depicting study participants with baseline and follow-up developmental assessments.

Figure 2. Forest plot summarizing cofactors for baseline developmental functioning (n=74).

Linear regression coefficients (95% confidence intervals) are denoted by black markers (horizontal black lines and whiskers). Circle, diamond, square and triangle markers represent gross motor, fine motor, social, and language domains, respectively.

Figure 3. Forest plot summarizing cofactors for 6-month change in developmental z-scores for baseline cofactors (A) and 6-month cofactors (B) adjusting for baseline developmental z-scores, child sex, and caregiver years of education (n=58). Analyses of change variables (CD4%, WAZ, HAZ, WHZ, and HCZ) are also adjusted by their baseline counterpart. Linear regression coefficients (95% confidence intervals) are denoted by black markers (horizontal black lines and whiskers). Circle, diamond, square and triangle markers represent gross motor, fine motor, social, and language domains, respectively.

LIST OF SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1. Table. Summary of univariate analyses of cofactors for baseline developmental z-scores (n=74) .doc

Supplemental Digital Content 2. Table. Summary of multivariate analyses of cofactors for 6-month change in developmental z-score (n=58) .doc

Table 1. Baseline characteristics of children with baseline developmental assessment
(n=74)

Baseline characteristics*	Median (25 th , 75 th percentiles) or n (%)
Child characteristics	
Age (years)	1.7 (0.8, 2.4)
Female	34 (46)
Pre-term birth	7 (9)
Ever breastfed	73 (100)
Received PMTCT [†]	13 (19)
WAZ	-2.14 (-3.93, -1.04)
Underweight (WAZ <-2)	40 (54)
HAZ	-2.34 (-3.49, -1.18)
Stunting (HAZ <-2)	43 (58)
WHZ	-1.55 (-3.38, -0.09)
Wasting (WHZ <-2)	31 (42)
HCZ	-0.53 (-1.49, 0.50)
Microcephaly (HCZ <-2)	9 (13)
CD4 T cell percentage	18 (11, 23)
CD4 T cell percentage <15%	28 (38)
WHO Stage 3 or 4	45 (62)
Blood hemoglobin (g/dL)	8.7 (7.5, 9.8)
Plasma HIV RNA (log ₁₀ c/ml)	5.6 (5.0, 6.2)

Child ART initiation characteristics

Randomized to ART at <48 hours 31 (42)

Initial ART Regimen:

ABC, 3TC, EFV 15 (20)

ABC, 3TC, LPV/r 38 (51)

ABC, 3TC, NVP 21 (28)

Time from ART initiation to developmental
assessment (days) 28 (27, 28)

Caregiver or household characteristics

Age (years) 27 (24, 31)

Biological mother 69 (93)

Married 46 (62)

Education (years) 8 (7, 11)

One-room house 28 (38)

Number of people per room 3 (2, 4)

Residing in Kisumu catchment 45 (60.8)

* n ≥70 for all variables except received PMTCT, n=69.

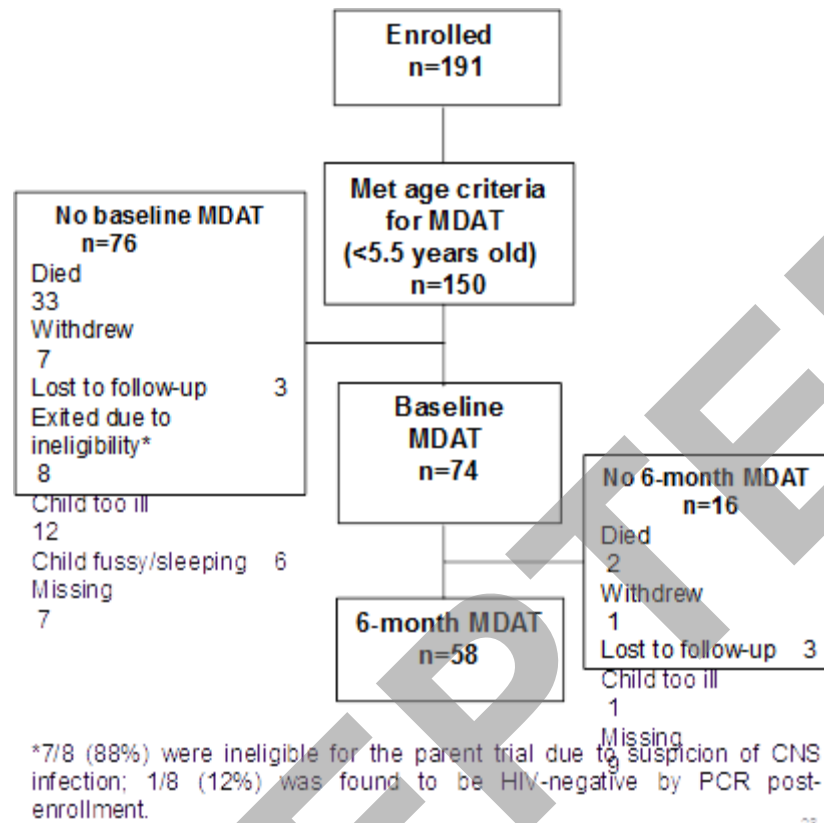
† Provided to mother only (n=2), provided to infant only (n=4), provided to both (n=7)

Table 2. Mean baseline and 6-month developmental z-scores (N=58)

	Baseline		6-month	
	N	Mean (SD)	Mean (SD)	P*
Gross motor	54	-1.05 (1.36)	-0.66 (1.38)	0.007
Fine motor	53	-1.03 (1.31)	-0.60 (1.15)	0.001
Social	57	-0.49 (1.08)	-0.38 (1.02)	0.4
Language	53	-0.89 (0.99)	-1.09 (1.03)	0.03

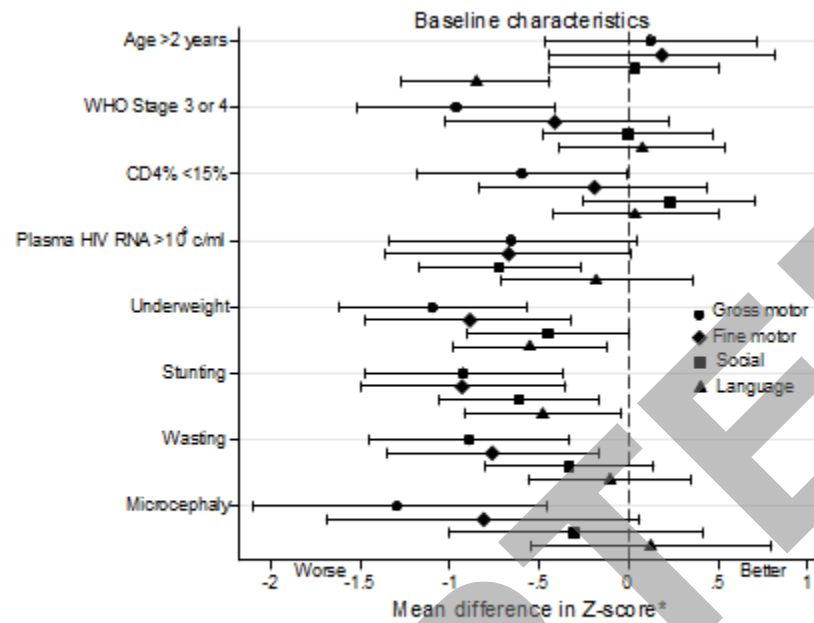
*Paired t-test.

Figure 1



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Figure 2



In sensitivity analyses excluding children with pre-term birth, results were similar, however the relation between underweight at baseline and lower social scores was no longer significant (mean difference, -0.45 vs -0.31, $P=0.17$).

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Figure 3

